What is the purpose of this form?

This application is to seek *initial* IRB approval for a research study.

What parts of this application should you submit?

Answer all questions, or mark "not applicable," when appropriate. Do not alter wording or delete questions from this form.

- For ***all studies***, submit Part A, which consists of these sections:
  - Part A.1. Contact Information, Agreements, and Signatures
  - Part A.2. Summary Checklist
  - Part A.3. Conflict of Interest Questions and Certification
  - Part A.4. Questions Common to All Studies
  - Part A.5. The Consent Process and Consent Documentation (including Waivers)

- For ***studies that involve direct interaction*** with human subjects (any contact with subjects including questionnaires, interviews, focus groups, observation, treatment interventions, etc), submit:
  - Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

- For ***studies*** that use existing data, records or human biological specimens, including for use in identifying potential subjects, submit:
  - Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

Note: You should submit Parts B or C only as applicable. If the study involves *both* direct interaction *and* use of existing materials, use both Parts B and C in addition to Part A.

Who can serve as principal investigator (PI)?

The PI is the person who will personally conduct or supervise this research study. Under most circumstances, this will be a faculty member. For IRB communication purposes, a trainee/student may be listed as PI. However, a faculty advisor must be identified, who holds ultimate responsibility for ensuring that this project complies with all University, regulatory, and fiscal requirements.

→ *See next page for additional instructions*

Complete submission instructions can be found at http://ohre.unc.edu/submission_instructions.php. **All application and consent materials must be copied or printed on one side only**. See the checklist on page 1 of the application itself for items to include and number of copies.

Some applications require additional review prior to the IRB submission. Examples include the Clinical and Translational Research Center (formerly the GCRC and CCCT facilities) http://gcrc.med.unc.edu/investigators/admin/gcrcapp.htm) or the Oncology Protocol Review Committee (PRC; http://cancer.med.unc.edu/research/prc/default.asp). See their web sites for details.

Many schools, departments, centers and institutes in Academic Affairs have local review committees that review before the IRB. See http://ohre.unc.edu/submission_instructions.php for a list of these units or consult your own unit for details.

Address for all Applications and Other Correspondence

IRB
CB# 7097, Medical Building 52
105 Mason Farm Road
Chapel Hill, NC 27599-7097

Types of Review

There are three levels of IRB Review (full board, expedited, and exempt), determined by the nature of the project, level of potential risk to human subjects, and the subject population. *The type of review applicable to a particular study is determined by the IRB.* Regardless of the kind of review, all applications use the same submission form.

Exempt and expedited review can be given to studies that constitute no more than minimal risk to the human subjects, i.e., the risk one experiences in daily living. These reviews are done in the IRB office on a continual basis.

Full board review is required for studies that involve greater than minimal risk or vulnerable populations that require special protection by the IRB. These require review by the convened IRB. See http://ohre.unc.edu/guide_to_irb.php for additional guidance.

---- Instructions – Do not submit this page with your application ----

OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version June 25, 2009

Part A.1. Contact Information, Agreements, and Signatures

Date: August 4th, 2011

IRB Study #09-1625

Title of Study: Epigenetic effects of diesel exhaust and ozone exposure

Name and degrees of Principal Investigator: David Diaz-Sanchez, Ph.D.; Kelly Duncan, Ph.D.
Department: US EPA Mailing address/CB #: 104 Mason Farm Road
Chapel Hill, NC 27514
UNC-CH PID: [redacted] Pager:
Phone #: 919-966-0676 Fax #: 919-966-6271
Email Address: Diaz-Sanchez.David@epa.gov, Duncan.kelly@epa.gov

For trainee-led projects: __ undergraduate __ graduate _ _ postdoc __ resident __ other
Name of faculty advisor:
Department: Mailing address/CB #:

Phone #: Fax #: Email Address:

Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any): Michael Schmitt, MSPH
Department: US EPA Mailing address/CB #: 104 Mason Farm Road
Chapel Hill, NC 27514
Phone #: 919-966-0647 Fax #: 919-966-6271 Email Address: Schmitt.mike@epa.gov

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include name, location (UNC or specific outside location), role and email address for each person who should receive electronic copies of IRB correspondence to PI.**

Robert Devlin, PhD.; Heidi Hiers, RN; Maryann Bassett, RN; Martin Case, BS; Andrew Ghio MD; Martha Sue Carraway, MD; Robert Truckner, MD;Howard Kehrl, MD; Tracey Montilla, RN; Michael Schmitt, MSPH, Sarah Snyder, B.S.; Melanie J. Jardim, Ph.D, Kristen Sanders, B.S.

Name of funding source or sponsor (*please do not abbreviate*):
__ not funded _X_ Federal __ State __ industry __ foundation __ UNC-CH
__ other (specify):

For external funding, RAMSeS proposal number (from Office of Sponsored Research): The PI is a USEPA scientist and this project is being funded by intramural EPA funds

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

Applications must "stand alone" and should provide all information requested, i.e., complete answers must be contained in the application. While you may reference other documents with supporting information, do not respond solely by stating "see attached."

Applications will be returned if these instructions are not followed.

Check	Item	Total No. of Copies
□	1. This application. One copy must have original PI signatures.	3
□	2. Consent and assent forms (include DHHS-approved sample, when one exists), fact or information sheets, phone and verbal consent scripts.	3
□	3. HIPAA authorization addendum to consent form.	3
□	4. All recruitment materials including final copies of printed advertisements, audio/video taped advertisements, scripts, flyers, letters, and emails.	3
□	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
□	6. Documentation of reviews from any other committees (e.g., Clinical and Translational Research Center (CTRC), Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
□	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
□	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
□	9. Data use agreements (may be required for use of existing data from third parties).	1
□	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
□	11. For drug studies, Investigator Brochure if one exists. If none, include package insert for previously approved uses..	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

Signature of Faculty Advisor Date

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

Signature of Department Chair or designee Date

Print Name of Department Chair or designee Department

Part A.2. Summary Checklist *Are the following involved?*	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	_X_
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	X__	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_X_
A.2.4. Do you have specific plans to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH employees?	_X_	__
b. Non-English-speaking?	__	_X_
c. Decisionally impaired?	__	_X_
d. Patients?	_X_	__
e. Prisoners, others involuntarily detained or incarcerated, or parolees?	__	_X_
f. Pregnant women?	__	_X_
g. Minors (less than 18 years)? ***If yes***, give age range: to years	__	_X_
A.2.5. a. Are sites outside UNC-CH engaged in the research?	__	_X_
b. Is UNC-CH the sponsor or lead coordinating center for a multi-site study?	__	_X_
If yes, include the ***Addendum for Multi-site Studies***.		
If yes, will any of these sites be outside the United States?	__	__
If yes, is there a local ethics review committee agency with jurisdiction? (provide contact information)	__	__
A.2.6. Will this study use a data and safety monitoring board or committee?	__	_X_
If yes: UNC-CH School of Medicine DSMB? (must apply separately)	__	__
Lineberger Cancer Center DSMC?	__	__
Other? Specify:	__	__
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	__	_X_
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__	_X_
c. Is this research classified (e.g., requires security clearance)?	__	_X_
A.2.8. a. Investigational drugs? (provide **IND #**)	__	_X_
b. Approved drugs for "non-FDA-approved" conditions?	__	_X_
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A.2.9. Placebo(s)?	__	__X
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #**)	__	_X_
A.2.11. Fetal tissue?	__	_X_
A.2.12. Genetic studies on subjects' specimens?	_X_	__
A.2.13. Storage of subjects' specimens for future research?	_X_	__
If yes, *see instructions for* ***Consent for Stored Samples***.		
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise?	__	__
If yes, *approval by the* ***UNC-CH Radiation Safety*** ***Committee*** *is required.*		X
A.2.15. Recombinant DNA or gene transfer to human subjects?	__	_X_
If yes, *approval by the* ***UNC-CH Institutional Biosafety*** ***Committee*** *is required.*		
A.2.16. Does this study involve UNC-CH cancer patients?	__	_X_
If yes, *submit this application directly to the* ***Oncology Protocol Review Committee***.		
A.2.17. Will subjects be studied in the Clinical and Translational Research Center (CTRC) or is the CTRC involved in any other way with this study? If yes, obtain the ***CTRC Addendum*** *and submit completed application (IRB application and Addendum) directly to the CTRC. The CTRC includes facilities located on the 3rd floor of the Main Hospital (formerly GCRC) and Ground floor Burnett-Womack (formerly CCCT).*	__	_X_
A.2.18. Will gadolinium be administered as a contrast agent?	..__	..__X
A.2.19. Will subjects' Social Security Number (SSN) be collected for:		
a. processing payments greater than $200 per year, to support IRS reporting (see also B.6)?	__	_X_
b. processing payments of any amount through UNC-CH Accounts Payable?	__	_X_
c. use as a unique identifier for study tracking purposes for national registry or database?	__	_X_

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	_X_ no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X_ no
(c) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity engaged in the performance of this project as a subcontractor, sub-recipient or vendor?	__ yes	_X_ no
(d) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X_ no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_X_ no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X_ no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit the form, which is accessible online at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

_____ _____

Signature of Faculty Advisor Date

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

Complete answers must be provided. While you may reference other documents with supporting information, do not respond solely by stating "see attached."

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: The purpose of this protocol is to compare the genetic and epigenetic effects between diesel exhaust and ozone exposure in healthy individuals and in mild/moderate asthmatics.

Participants: We will recruit up to 30 mild to moderate asthmatics and up to 50 healthy adults to participate in this study.

Procedures (methods): Subjects will be exposed to clean air, to 300 µg/m^3 of diesel exhaust for 2 hours and to 0.3 ppm of ozone for 2 hours with intermittent exercise in a controlled environment chamber. Primary endpoints will include spirometry and lung cell changes post-exposure. Secondary endpoints will include analysis of blood clotting/coagulation factors, Holter monitoring of cardiac parameters, analysis of soluble factors present in plasma and bronchial lavage and analysis of intracellular factors present in lung tissue obtained from a brush biopsy.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Several epidemiological studies have shown a strong link between exposure to air pollution and adverse cardiopulmonary effects, such as respiratory tract infections, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke(Brunekreef and Holgate 2002; Riedl and Diaz-Sanchez 2005; Lewtas 2007). Ozone and diesel exhaust are ubiquitous in the urban atmosphere making it difficult to identify the exact source of these effects. Although experimental chamber studies have been previously performed with diesel exhaust or ozone, no one has yet looked at the epigenetic changes resultant from acute exposures to these air pollutants.

Numerous studies have shown that healthy subjects exposed to ozone have decreased pulmonary function and increased neutrophils and soluble pro-inflammatory mediators in the lower airways(Peden, Setzer et al. 1995; Bascom 1996). These include markers of increased permeability, decreased macrophage phagocytic ability, and increases in arachidonic acid metabolites, cytokines, fibronectin, lactase dehydrogenase, coagulation factor VII, tissue factor, elastase and plasminogen activator occurred in response to ozone-induced exposures(Devlin, McDonnell et al. 1991; Koren, Devlin et al. 1991; McDonnell, Kehrl et al. 1991; Devlin 1993; McKinnon, Madden et al. 1993). Diesel exhaust fumes contain primarily fine and ultrafine carbonaceous particles generated by the incomplete combustion of fuel(Diaz-Sanchez, Penichet-Garcia et al. 2000; Lewtas 2007). Diesel exhaust particles (DEP) are the largest single source of vehicular emitted airborne PM and can persist in the air, where they are readily inhaled and deposited throughout the respiratory tract. DEP induced toxicity is mainly attributed to its chemical composition, consisting of a carbonaceous core to which organic and inorganic species can become adsorbed (Brunekreef and Holgate 2002; Riedl and Diaz-Sanchez 2005). Healthy human volunteers exposed to 300 µg/m^3 diesel exhaust for one hour with intermittent exercise resulted in markers of systemic and redox-sensitive pulmonary inflammatory responses, vascular dysfunction and impaired endogenous fibrinolysis. Although there were no adverse health effects associated with the exposure, acute changes in the aforementioned endpoints can be used to extrapolate information in order to begin to understand the underlying biological mechanisms that link long-

term exposure of diesel exhaust inhalation to the pathogenesis of atherothrombosis and acute myocardial infarction (Salvi, Blomberg et al. 1999; Behndig, Mudway et al. 2006).

Previous studies reported a wide variation in response to air pollution exposure among individuals in each subject group with no clear understanding in reasons (McDonnell 1991). Asthmatics have been identified as a susceptible subpopulation as seen by their heightened sensitivity to air pollutants, although the severity of health responses of asthmatics exposed to diesel exhaust and ozone are not yet fully understood. It is hypothesized that increased susceptibility could be due to increased baseline airway inflammation and airway responsiveness which characterizes asthma. Indeed, respirable particulate matter and ozone exposure are associated with episodes of increased asthma exacerbation (Devlin 1993; Bascom 1996; Diaz-Sanchez and Riedl 2005). Elevated levels of air pollution can affect airway reactivity with decrements in several indices of pulmonary function especially in those patients with preexisting asthma.

Despite a decade of intensive studies, adverse health effects attributed to air pollutant exposure are still not well understood. It is becoming increasingly clear that epigenetics plays a pivotal role in disease development and outcome, which has been extensively studied in cancer. The term epigenetics refers to the study of heritable gene expression changes, which occur without changing the DNA sequence itself. DNA methylation, histone modifications and microRNA expression all regulate gene expression, and changes in these mechanisms can result in an overall change in gene expression patterns. Unlike the static DNA sequence, these mechanisms are regulated by endogenous and exogenous cues, rendering them more likely to change in response to environmental stimuli. It is not currently known how these changes become permanent, or how epigenetic changes control gene expression in response to acute environmental perturbations. Few studies have examined how the environment can modulate epigenetic marks in individuals. In a pioneering study published in 2005, Fraga et. al. showed that as identical twins age, DNA methylation patters change indicating that differential gene expression observed in older identical twins may be attributed to epigenetic changes, further suggesting that these changes may have been molded by the environment (Fraga, Ballestar et al. 2005). Traffic particle exposure has been associated with methylation changes in retrotransposable elements, randomly dispersed throughout the DNA sequence, however the biological consequence of this is not understood as these are not coding sequences (Baccarelli, Wright et al. 2009). In vitro studies in primary human bronchial epithelial cells grown at air liquid interface showed that microRNA expression profiles change rapidly in response to exposure to diesel exhaust particles(Jardim 2009). Animal studies attempting to address these questions have been difficult as animals have very different epigenetic mechanisms making it difficult to draw conclusions from these studies. Long term exposure to cigarette smoke, a source of particulates, lead to changes in microRNA expression in rat lungs, however the contribution of these changes to biological consequences is not currently known (Izzotti, Calin et al. 2009). Another study, showed that an in utero diet rich in methyl donors enhanced allergic airway disease in mice via changes in methylation of several genes, and that these changes were transgenerational (Hollingsworth, Maruoka et al. 2008). While it is difficult to draw biological meaning from epigenetic animal studies, what these studies show is that the environment can play an important role in regulating gene expression. Although the biological and physiological role epigenetics plays in response to environmental pollutants is not yet understood, however we will begin to address this question in this study by performing in depth gene expression analysis, including microarrays, microRNA arrays and DNA methylation assays on samples collected from patients.

In addition to possible epigenetic mechanisms, genetic polymorphisms, particularly polymorphisms in anti-oxidant genes, have been proposed as a factor to explain variability in these responses. The role of genetic background in mediating airway and vascular inflammation following acute exposure to diesel exhaust and ozone in human subjects will be studied in this protocol as a secondary question. As such, bronchial epithelial cells collected from this study will be further evaluated by in vitro exposure to both gaseous and particulate air pollution.

The purpose of this study is to first, make a direct comparison of epigenetic responses (primarily changes in DNA methylation, microRNA expression and chromatin modification) in individuals exposed to ozone and diesel exhaust; second, to assess whether mild to moderate asthmatics exhibit the similar effects and to the same degree as healthy individuals; and third, to understand the contribution of genetic background and epigenetic mechanisms to cardiopulmonary effects observed in pollutant exposed cells. Although many the observed adverse health effects are generally attributed to oxidative stress upon exposure, we still do not know how these result in physiological changes to the respiratory and cardiovascular organ systems. We hypothesize that exposure to diesel exhaust or ozone will result in changes in epigenetic mechanisms, which may be linked to adverse health effects of exposure. If adverse health effects are induced solely due to oxidative stress, then patients should exhibit similar cardiopulmonary and epigenetic responses when exposed to either, diesel exhaust or ozone. However, if differential

responses to these agents are observed, then alternative mechanisms of cellular injury may exist. Therefore, we will employ several methods to assess potentially important physiological changes to these systems, including cutting edge techniques such as micro-array profiling, DNA methylation assays, microRNA expression profiling and assays for indications of chromatin remodeling.

Baccarelli, A., R. O. Wright, et al. (2009). "Rapid DNA methylation changes after exposure to traffic particles." Am J Respir Crit Care Med **179**(7): 572-8.

Bascom, R. (1996). "Environmental factors and respiratory hypersensitivity: the Americas." Toxicol Lett **86**(2-3): 115-30.

Behndig, A. F., I. S. Mudway, et al. (2006). "Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans." Eur Respir J **27**(2): 359-65.

Brunekreef, B. and S. T. Holgate (2002). "Air pollution and health." Lancet **360**(9341): 1233-42.

Devlin, R. B. (1993). "Identification of subpopulations that are sensitive to ozone exposure: use of end points currently available and potential use of laboratory-based end points under development." Environ Health Perspect **101 Suppl 4**: 225-30.

Devlin, R. B., W. F. McDonnell, et al. (1991). "Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung." Am J Respir Cell Mol Biol **4**(1): 72-81.

Diaz-Sanchez, D., M. Penichet-Garcia, et al. (2000). "Diesel exhaust particles directly induce activated mast cells to degranulate and increase histamine levels and symptom severity." J Allergy Clin Immunol **106**(6): 1140-6.

Diaz-Sanchez, D. and M. Riedl (2005). "Diesel effects on human health: a question of stress?" Am J Physiol Lung Cell Mol Physiol **289**(5): L722-3.

Folinsbee, L. J., J. F. Bedi, et al. (1985). "Pulmonary response to threshold levels of sulfur dioxide (1.0 ppm) and ozone (0.3 ppm)." J Appl Physiol **58**(6): 1783-7.

Fraga, M. F., E. Ballestar, et al. (2005). "Epigenetic differences arise during the lifetime of monozygotic twins." Proc Natl Acad Sci U S A **102**(30): 10604-9.

Hollingsworth, J. W., S. Maruoka, et al. (2008). "In utero supplementation with methyl donors enhances allergic airway disease in mice." J Clin Invest **118**(10): 3462-9.

Izzotti, A., G. A. Calin, et al. (2009). "Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke." FASEB J **23**(3): 806-12.

Jardim, M., Fry, RC, Jaspers, I, Dailey, L and Diaz-Sanchez, D (2009). "Disruption of MicroRNA Expression in Human Airway Cells by Diesel Exhaust Particles is Linked to Tumorgigenesis-Associated Pathways." Environmental Health Perspectives **117**(11).

Koren, H. S., R. B. Devlin, et al. (1991). "Time-dependent changes of markers associated with inflammation in the lungs of humans exposed to ambient levels of ozone." Toxicol Pathol **19**(4 Pt 1): 406-11.

Lewtas, J. (2007). "Air pollution combustion emissions: characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects." Mutat Res **636**(1-3): 95-133.

McDonnell, W. F. (1991). "Intersubject variability in human acute ozone responsiveness." Pharmacogenetics **1**(2): 110-3.

McDonnell, W. F., H. R. Kehrl, et al. (1991). "Respiratory response of humans exposed to low levels of ozone for 6.6 hours." Arch Environ Health **46**(3): 145-50.

McKinnon, K. P., M. C. Madden, et al. (1993). "In vitro ozone exposure increases release of arachidonic acid products from a human bronchial epithelial cell line." Toxicol Appl Pharmacol **118**(2): 215-23.

Peden, D. B., R. W. Setzer, Jr., et al. (1995). "Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics." Am J Respir Crit Care Med **151**(5): 1336-45.

Riedl, M. and D. Diaz-Sanchez (2005). "Biology of diesel exhaust effects on respiratory function." J Allergy Clin Immunol **115**(2): 221-8; quiz 229.

Salvi, S., A. Blomberg, et al. (1999). "Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers." Am J Respir Crit Care Med **159**(3): 702-9.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified. Researchers are reminded that additional approvals may be needed from relevant "gatekeepers" to access subjects (e.g., school principals, facility directors, hospital or healthcare system administrators).

Subjects for this study will be healthy adults, 18-55 years old, (male and female) and, otherwise healthy, 18-55 year old mild to moderate asthmatics (as defined by NHLBI guidelines), who are non-smoking. Subjects will be recruited through the Westat Corporation (see section B1 below). There are no gender or race based restrictions. Pregnant and lactating women will be excluded because of the unknown effects of air pollutants on inflammatory markers during pregnancy and lactation.

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Healthy Individuals

Specific Inclusion Criteria

1. Physical exams will be performed by study physicians during the screening visit (IRB approved EPA protocol 95-EPA-66).
2. Normal lung function (pre or post albuterol), defined by Knudson 1976/1984 as:
   a. FVC of $\geq$ 75 % of that predicted for gender, ethnicity, age and height.
   b. FEV_1 of $\geq$ 75 % of that predicted for gender, ethnicity, age and height.
   c. FEV_1/FVC ratio of $\geq$ 75 % of predicted values.
3. Oxygen saturation of $\geq$ 96 %.
4. Ability to tolerate intervals of moderate exercise

Exclusion Criteria:

1. A history of chronic illnesses such as diabetes, rheumatological diseases, immunodeficiency state, known clinically significant cardiac disease (including myocardial infarction, congestive heart failure and angina), chronic respiratory diseases such as chronic obstructive pulmonary disease, and lung cancer.
2. If the subject is pregnant, attempting to become pregnant or breastfeeding.
3. Allergy to any medications which may be used or prescribed in the course of this study.
4. Subjects currently taking mega doses of vitamins and supplements, homeopathic/naturopathic medicines or medications which may impact the results of the PM and/or ozone challenge or interfere with any other medications potentially used in the study (to include systemic steroids and beta blockers). Subjects must refrain from all over the counter anti-inflammatory agents including those for allergies, and naproxen, and anti-oxidants for a period of one week prior to exposure. Medications not specifically mentioned here may be reviewed by the investigators prior to a subject's inclusion in the study.
5. Smoking history within 2 years of the study.
6. Use of inhaled steroids, cromolyn, or leukotriene inhibitors (Montelukast, Zafirkulast, etc) initiated within the past month (except for use of cromolyn exclusively prior to exercise). Patients must be on a stable regimen of therapy.
7. Untreated hypertension (> 150 systolic, > 90 diastolic)
8. Dementia.
9. Unspecified illnesses, which in the judgment of the investigator might increase the risk associated with PM inhalation challenge or exercise, will be a basis for exclusion.
10. History of skin allergy to tape or electrodes.
11. Subjects who do not understand or speak English
12. Subjects who are unable to perform moderate exercise

Temporary exclusions:

1. Viral upper respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of bronchoscopy.
2. Use of daily medications(at the discretion of physician)

3. Symptom score greater than 20 (out of a possible 60-see accompanying score sheet) for total symptom score with a value greater than 3 for any one score. Only one score may be equal to 3.
4. Current exacerbation of allergic rhinitis and or use of antihistamines during one week prior to exposure.
5. Recent or recurring exposure to pollutants or irritants

Exclusion criteria for bronchoscopy:

1. Any food or fluids after midnight prior to bronchoscopy
2. FEV1/FVC ratio less than 60% predicted on AM of bronchoscopy.
3. Regular use of aspirin ≥ 81 mg daily, or other nonsteroidal anti-inflammatory drugs (which inhibit platelet function).

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

Mild to Moderate Asthmatics

Inclusion Criteria:

1. Physical exams will be performed by study physicians during the screening visit (IRB approved EPA protocol 95-EPA-66) where diagnosis will be judged by EPA physicians.
2. Mild/Moderate asthmatics as defined by NHLBI guidelines.
3. Positive history of asthma (wheezing, chest tightness, and reversible airway obstruction);
4. Baseline $FEV_1/FVC \geq 60\%$;
5. Oxygen saturation of ≥ 94%
6. Ability to tolerate moderate exercise

Exclusion Criteria

1. Use of oral steroid therapy within the past month
2. Physician directed emergency treatment for asthma exacerbation within the preceding 6 months.
3. Abnormal EKG that precludes evaluating heart rate variability.
4. Aside from mild/moderate asthma, a history of chronic illnesses such as diabetes, rheumatological diseases, immunodeficiency state, known clinically significant cardiac disease (including myocardial infarction, congestive heart failure and angina), chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma, and cancer (possible exception for history of nonmelanoma skin cancer).
5. If the subject is pregnant, attempting to become pregnant or breastfeeding.
6. Allergy to any medications which may be used or prescribed in the course of this study
7. Subjects currently taking mega doses of vitamins and supplements, homeopathic/naturopathic medicines or medications which may impact the results of the PM challenge or interfere with any other medications potentially used in the study (to include systemic steroids and beta blockers). Subjects must refrain from all over the counter anti-inflammatory agents including those for allergies, and naproxen, and anti-oxidants for a period of one week prior to exposure. Medications not specifically mentioned here may be reviewed by the investigators prior to a subject's inclusion in the study.
8. Dosing level of an inhaled steroid must be consistent with mild asthma as outlined by the NHLBI NAEPP guidelines. Regular use of oral corticosteroids, or use of inhaled steroid at doses typically used for severe asthma, will result in exclusion of that individual from the protocol.
9. Severe asthmatics as defined by: nighttime symptoms of cough or wheeze greater than 1 time per week at baseline, daily exacerbation of asthma or requirement for albuterol due to asthma symptoms (cough, wheeze, chest tightness, but not to include prophylactic use of albuterol prior to exercise), more than mild interference with normal activity, any episode of physician directed emergency treatment for asthma requiring oral corticosteroid therapy within the past twelve months.
10. Smoking history within 2 years of study.
11. Use of inhaled steroids, cromolyn, or leukotriene inhibitors (Montelukast, Zafirkulast, etc) initiated within the past month (except for use of cromolyn exclusively prior to exercise). Patients must be on a stable regimen of therapy.
12. History of skin allergy to tape or electrodes.
13. History of respiratory diseases other than allergic rhinitis and asthma
14. Untreated hypertension (> 150 systolic, > 90 diastolic)
15. Dementia.

16. Unspecified illnesses, which in the judgment of the investigator might increase the risk associated with PM inhalation challenge or exercise, will be a basis for exclusion.
17. Subjects who do not understand or speak English
18. Subjects who are unable to perform moderate exercise

Allowances: subjects may
1. Use daily theophylline therapy.
2. Use daily inhaled steroids.
3. Use inhaled cromolyn therapy.

Temporary exclusions:
1. Viral upper respiratory tract infection or any acute infection requiring antibiotics within 6 weeks of bronchoscopy.
2. Use of daily medications(at the discretion of physician)
3. Symptom score greater than 20 (out of a possible 60-see accompanying score sheet) for total symptom score with a value greater than 3 for any one score. Only one score may be equal to 3.
4. Current exacerbation of allergic rhinitis and or use of antihistamines during one week prior to exposure.
5. Recent or recurring exposure to pollutants or irritants

Exclusion criteria for bronchoscopy:
1. Any food or fluids after midnight prior to bronchoscopy
2. FEV1/FVC ratio less than 60% predicted on AM of bronchoscopy.
3. Regular use of aspirin ≥ 81 mg daily, or other nonsteroidal anti-inflammatory drugs (which inhibit platelet function).

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

This is a randomized cross-over study with three arms. This study will compare the epigenetic responses of healthy adults and adults with mild asthma to diesel exhaust and ozone exposures. Up to fifty subjects will be recruited into the healthy adult group and up to thirty subjects into the mild to moderate asthmatic group. Subjects will be randomly exposed to clean air, diesel exhaust and ozone with exposures separated by a minimum of two weeks. Responses of primary interest will include: 1) genetic and epigenetic changes 2) FEV1 as measured by spirometry, and 3) lung inflammation and cell changes as evaluated by bronchoalveolar lavage. The performance of these tests and procedures are considered essential to conducting the study. Exploratory endpoints will include: blood CBC and differential, fibrinogen and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, telemetry, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18-24 hours post-exposure. All medical procedures will be performed by study personnel.
Up to an additional 15 healthy subjects will be recruited specifically to be exposed to ozone generated using the heavy non-radioactive isotope of oxygen (18O). There is no risk to the subject of inhaling this isotope, which is naturally occurring in small concentrations. We have previously used 18Ozone in a study approved by the UNC IRB in 1993. The purpose of these exposures is to measure the amount of 18O label attached to airway epithelial cells removed during bronchoscopy and thus calculate the dose of ozone these cells received. We will draw a small

blood sample but no other tests will be performed on these subjects other than routine spirometry to ensure they are not at risk for bronchoscopy.

Physical Examination Day:

Prior to recruitment into the study, subjects will undergo a physical exam. During this visit, a short review of the subject's history will be performed and vital signs, height and weight will be assessed (temperature, pulse, respiratory rate, and blood pressure), to determine whether the individual meets any major exclusion criteria for the study. Up to 50 ml of blood may be collected for a CBC/ differential, chemistry and lipid panels. At this time the subject may undergo a physical examination for bronchoscopy or may be scheduled for one at another time.

In order to participate in this study, subjects will be asked to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- On the Exposure Day 2, eat a normal breakfast.
- No caffeine for 12 hours prior to all study visits.
- At the end of Exposure Day 2, do not eat or drink anything after midnight in preparation for bronchoscopy on Exposure Day 3.

Training Day

1. Eligible subjects will report to the research lab for an approximate 2-hour training session.
2. Informed consent will be obtained.
3. Assessment of **vital signs** (temperature, pulse, respiratory rate, and blood pressure), oxygen saturation
4. **Pregnancy tests** will be administered to any women who may have child-bearing potential**.**
5. Subjects will be trained on the cycle ergometer and the work load to elicit a minute ventilation normalized for body surface of 25L/m^2/BSA will be determined. In most subjects this will be about 50 L/minute (IE. VO^2 of approximately 2.0 L/m). A cycle ergometer work setting of 75 to 100 watts will usually achieve such a physiological response
6. Subjects will perform **spirometry.**
7. Subjects will be shown chamber where exposures will take place.

Exposure Day 1

The subjects will be exposed ozone, diesel exhaust and clean air on three separate occasions, with the exposures separated by a minimum of two weeks. Subjects receiving 18Ozone, will only be exposed to ozone.

1. Subjects will be asked to arrive at the study site no sooner than 8 AM and will undergo **assessment of vital signs** (temperature, pulse, respiratory rate, and blood pressure), oxygen saturation, and **symptom score** assessment.
2. **Pregnancy tests** will be administered to any women who may have child-bearing potential**.**
3. A **telemetry unit** will be attached for cardiac monitoring.
4. Subject will be fitted with a **Holter monitor** and the subject will be asked to recline in a quiet dark place for 30 minutes. After approximately 20 minutes a time marker will be activated so that heart rate variability frequency readings can be taken over the next 10 minutes. The subject will wear the Holter monitor for the next 24 hrs. Subjects exposed to 18Ozone will not be fitted with a Holter.
5. **Venipuncture:** Up to 80 ml of blood will be collected for a CBC/ differential, and may be used for assessment of coagulation function and markers, phagocytic function, inflammation, and cell surface markers. A portion of the sample may be used for RNA isolation for microarray analysis and for DNA isolation for genotyping purposes. Pre-exposure blood will not be taken from subjects being exposed to 18Ozone
6. **Baseline Spirometry**
7. **Exposure sessions**: Exposure to ozone, diesel exhaust or clean air will be conducted in an exposure chamber at the EPA Human Studies Facility on the UNC campus. Each subject will be exposed up to 0.3ppm ozone, diesel exhaust (up to 300 ug/m^3) or clean air for 2 hours. Subjects will begin exercising on an exercise bike. Each exercise session will consist of a 15 minute exercise interval at a level of up to 25 L/m2/BSA followed by a 15 minute rest period. Minute ventilation may be measured during each exercise

period. Continuous heart rate and rhythm, and oxygen saturation, will be monitored using telemetry and pulse oximetry respectively. Blood pressure may be monitored intermittently. Subjects will be asked to refrain but not prohibited from using inhaled bronchodilator during exposures; if medication use is necessary subjects will be instructed to maintain consistent medication use across the two exposures. The subjects will be able to end their exposure and exit the chamber at any time. Indications for terminating the exposure include significant respiratory distress or dyspnea, chest or angina-like pain, significant cardiac arrhythmias, pallor, ataxia, or a greater than 5 point drop in saturated oxygen (or a drop to lower than 89%). They will be monitored continuously by trained personnel and a physician is on call whenever a subject is undergoing a procedure at the facility. A detailed description of the physical facilities in the Human Studies Division of the U.S. EPA Health Effects Research Laboratory located on the UNC-CH campus have been published as U.S. Government Publication EPA-600/1-78-064, and is on file in the office of the UNC Committee on the Protection of the Rights of Human Subjects. The medical station of the Human Studies Division includes a fully-equipped medical examination room, a cardiac/respiratory emergency cart, a subject recovery room, a nurse's station, a waiting room and conference rooms. The facility is staffed by qualified RNs, and an on-site physician is available to respond to a medical emergency in a timely manner**.** The exposure atmosphere will be at approximately 40 $\pm$ 10% RH and approximately 22 $\pm$ 2 ^{o}C. The DE will be generated from a diesel generator used to power a load bank that is located outside the Human Studies Facility, and subsequently introduced into the exposure chamber after different dilutions with clean HEPA and charcoal filtered and humidified air to give a chamber concentration of up to 300 $\mu g/m^3$. The monitoring analyzer registers a concentration in real time. Exposures will be terminated at values $\geq$ 400 $\mu g/m^3$ during runs in which the exposure targets 300 $\mu g/m^3$ DE. This safety limit will prevent an inhalational exposure greater than 1080 µg, which assumes that the subject will inhale 2.7 m^3 during the 120 min exposure. Levels of carbon monoxide (CO), and oxides of nitrogen (NOx; mainly NO, and some NO_2), will be kept under 10 ppm CO, 15 ppm NO, and 2.5 ppm NO_2 or the exposure will be terminated. [The OSHA 8 hr time-weighted average (TWA) for these substances are: CO=50 ppm; NO=25 ppm; NO_2=5 ppm; Diesel fuel used for the study will be purchased as a commercial ultra low sulfur fuel. Subjects will enter the exposure chamber (about 6 ft x 6 ft x 8 ft). Particle mass will be measured in real time. Particle size distribution may not be determined during each subject exposure but at regular intervals (e.g., monthly) to show that the size distribution does not change radically. Filter samples will also be analyzed for chemical composition of particles. Ozone exposures will be conducted in a (6 ft x 6 ft x 8 ft) stainless steel chamber with a continuous supply of exposure medium. Ozone will be monitored continuously. **Immediately post exposure**, subjects will again undergo **spirometry, Holter monitor frequency reading, symptom scoring and assessment of vital signs.**

8. **Then,** up to 80 ml of blood will be collected for a CBC/ differential, assessment of coagulation function, blood leukocyte phagocytic function, cytokines, cell surface markers and RNA isolation for microarray analysis. Subjects exposed to 18Ozone will have only 20 mls of blood removed for analysis of 18O tag present on blood cells and have post exposure Spirometry measurements.
9. **Subjects exposed to 18Ozone will have bronchoscopy performed about 1 hr post-exposure.** Exposure to 18Ozone and undergoing a bronchoscopy approximately one hour later should not increase the risk to the subjects. We have completed two earlier studies in which subjects underwent bronchoscopy following exposure to concentrations of ozone higher than used in this study, and encountered no problems. Others have also published studies in which subjects were exposed to ozone followed by bronchoscopy one hour later
10. Subjects will then be assessed and discharged by the nursing staff.

Exposure Day 2 (approximately 24 hours after exposure)
Subjects being exposed to 18O will not be returning for a Day 2

The subject will arrive at the U.S. EPA HSF for:

1. **Vital sign monitoring**.
2. **Symptom scoring**.
3. **Venipuncture**: up to 80 ml of blood will be collected for a CBC/ differential, coagulation function, assessment of blood monocyte phagocytic function, inflammation, and cell surface markers, and RNA isolation for microarray analysis.
4. **Holter monitor** frequency reading and removal
5. **Spirometry**

6. **Bronchoscopy.** Subjects will have undergone a physical examination including assessment for suitability for transnasal fiberoptic bronchoscopy at the Human Studies Facility by a board certified or board eligible pulmonologist.

Prior to the procedure, subjects will perform spirometry (as above); bronchoscopy will not be performed if lung function does not meet the recommended performance level (NIH guidelines; FEV1≥ 60 % predicted). Prior to bronchoscopy, a small tube (IV) will be placed in a vein for potential use in administering fluids. Subjects will receive inhaled albuterol prior to the bronchoscopy at the discretion of the pulmonary physician performing the procedure. Bronchoscopy with bronchoalveolar lavage and brush biopsy will be performed by a licensed physician who is Board certified or board eligible in pulmonary medicine and is experienced in fiberoptic bronchoscopy. The physician will be assisted by at least one R.N experienced in bronchoscopy. The subjects will be monitored closely throughout the procedure in the following manner: 1) Chest electrodes for continuous electrocardiogram, 2) Pulse oximetry of arterial blood, and 3) blood pressure monitoring using an electronic sphygmomanometer. Subjects may be premedicated with IV atropine (0.6 mg) to prevent bradycardia and hypotension that could result from vagal stimulation provoked by passing the bronchoscope through the subject's larynx and to minimize the amount of airways secretions. Nasal oxygen will be administered during the procedure. No sedatives and/or narcotics are administered at any time during bronchoscopy. We have found that premedication with sedatives (midazolam) or with opiates (demerol) is not required for research bronchoscopy subjects. Avoidance of these drugs reduces procedure risk and markedly shortens post-procedure recovery time. Neosynephrine will be used to decongest nasal passages, followed by gargling with a lidocaine solution for a few seconds to anesthetize the throat. The subject will then sniff (snort) a small amount of lidocaine jelly through one nostril to anesthetize the nose and the back of throat. A Q-tip with lidocaine jelly will be gently inserted into the nose to ensure that it is completely numb before the bronchoscope is inserted. Topical lidocaine anesthesia will be administered to the larynx and lower airways through the bronchoscope for subject comfort and to prevent cough, with a maximum dose of 15 ml of 2% lidocaine solution (360 mg lidocaine) Up to 8 endobronchial brush biopsies will be obtained from the lower trachea and right and left mainstem bronchi. A cytology brush (Bronchoscope Cytology Brush; Bard, Tewksbury, MA) is applied to the region under direct visualization. For a single brushing, six passes, each with a linear excursion of approximately 2-5 cm, are made with the brush along the endobronchial surface. Bronchoalveolar lavage will be performed in the right middle lobe using a total volume of up to 250 cc of sterile 0.9% saline; from one to 5 aliquots of 50 cc each will be injected and immediately aspirated through the channel in the bronchoscope using a syringe.

Subjects are observed and monitored for a minimum of 1 to 2 hours after the procedure in the on-site medical station by a registered nurse with physician supervision. At discharge subjects will be symptom-free. At least 2 physicians, including the responsible bronchoscopist, will be available (24 hours daily) to subjects who develop any symptoms after discharge. The subject is discharged by a physician and is provided with the names and telephone numbers of the bronchoscopist and one other physician. The subject is contacted 24 hours post-bronchoscopy to ask about any untoward effects.

Subjects will return to the U.S. EPA HSF for an additional exposure sessions and post exposure bronchoscopies (air control, ozone or diesel exhaust) after a minimum time interval of two weeks.

We anticipate performing several clinical procedures during the course of this study which include primary, secondary and exploratory endpoints. However, it is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure, because of a investigator problem (such as but not limited too building/equipment failures, weather delays, or staffing issues), then the patient will be compensated for said procedure(s) and time on that day and may be rescheduled.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

There will be no direct benefit to the subject for participating, other than information that may be learned during their physical examination about their overall physical status. The primary benefit from the study will be to add to the scientific knowledge about whether exposure to air pollutants induces epigenetic changes.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

Risk and Procedures for Minimizing Risks of Diesel Exhaust Exposure

- Diesel Exhaust Exposure: The amount of diesel exhaust used for exposure in this study would be equivalent to concentrations of diesel exhaust particulate matter that would be encountered at busy intersections in large urban area. These concentrations are the occupational levels for some truck drivers (generally about 100-300 $\mu g/m^3$) and 1-2 mg/m^3 for some mines. Some areas of heavily trafficked streets in Los Angeles and New York City have had diesel exhaust levels >20 $\mu g/m^3$, and nearby residents could have exposure to these concentrations over several hours. Controlled diesel exhaust exposure studies in Sweden using 300 $\mu g/m^3$ DEP have shown lung inflammatory effects not unlike effects observed with numerous ozone exposure studies performed here at the EPA facility on the UNC campus during the past 20 yr. Diesel exhaust particles contain some probable carcinogenic polycyclic aromatic hydrocarbons, which in high enough concentrations and/or with repeated exposures may induce tumors. Diesel exhaust also contains aldehydes, some of which are possibly carcinogenic in high enough dose and with long enough exposure. However, the exposure concentrations to be used in this protocol are minimal. Overall, it appears that at the low DEP concentration to be given one time for the exposure in this study, the risk of cancer, if it exists at all, is extremely low and certainly no more than what one would experience if one were to visit for a few days a particulate-polluted city in the US such as Los Angeles or New York City. We have seen in IRB-07-0190 that exposure to 300 $\mu g/m^3$of diesel exhaust does not cause adverse health effects.

Health effects of acute exposures to air pollution particles are not well defined but possibly include chest pain, mild dyspnea, headache, cough, and, in some subjects, wheezing. All of these effects resolve spontaneously within hours of exposure cessation. In the pilot phase of study #07-0190 (Haiyan Tong MD, Ph.D, PI), subjects reported "moderate" eye irritation following diesel exhaust exposure and the symptom was resolved within a few hours after the exposure. Subjects will be provided with the choice to wear chemical safety goggles during exposures to prevent or minimize the eye irritation. During exposure, subjects will be monitored by direct observation or via closed-circuit television. Subjects will have EKG leads attached during the exposure to monitor cardiac function during exercise. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for their participation up to that point. The investigators or duty physician will end the exposure if the subject is found to be suffering from any significant adverse effect. No major adverse health effects were observed in a recently completed study that exposed subjects (18-35 yr old, healthy volunteers) to concentrated Chapel Hill air particles here at the EPA facility (Physiological and Biochemical Changes Associated with Exposure to Air Pollution Particles; Andrew Ghio, MD, PI; UNC IRB# 95-EPA-310). The maximum PM concentration reached 311 $\mu g/m^3$ during the exposure. The data from this exposure study suggest that normal healthy subjects tolerate inhalation of concentrated ambient PM fairly well, and support the idea that exposures to lower PM concentrations, including DEP, will be fairly safe in terms of cardiopulmonary responses. For safety reasons, pulse oximetry will be performed during the exposures to diesel exhaust and filtered air, and the subject withdrawn from the chamber if the O_2 saturation value is <89%. Additionally subjects will have carboxyhemoglobin values determined within 1 hr after an exposure and kept at the Medical Station if levels are >20% (where performance of everyday cognitive tasks may be jeopardized) until levels become <20%; but because the CO levels are expected to be 2-4 ppm [based on preliminary chamber characterizations], levels are expected to be < 5%.

A duty physician is always onsite to respond to an emergency. Fully equipped resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. Physicians at the University of North Carolina (UNC) Hospitals Emergency Room are also available to assist in treatment of an emergency.

Heart rate, electrocardiogram, and pulse oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmias, pallor, and ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire exposure session. The investigator or duty physician will end the exposure if the subject is found to be suffering from any adverse effect.

Risk and Procedures for Minimizing Risks of Ozone Exposure

Potential risks may likely include mild airway obstruction, irritation to the nose, eyes, throat and airways, pain on deep inspiration and cough. These symptoms typically disappear 2 to 4 hours after exposure, but may last longer for particularly sensitive people. Ozone may induce an inflammatory reaction that may last for about 24-48 hours after exposure and may increase the chance of catching a cold. It is likely that most subjects participating in this study will experience some degree of tracheobronchial irritation and cough. There is no additional risk associated with the heavy isotope (18O) of ozone.

Risks and Procedures for Minimizing Risks of Venipuncture:

Insertion of the needle may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.

Risks and Procedures for Minimizing Risks of Bronchoscopy:

The most significant risk to a volunteer in this protocol involves bronchoscopy. Over 1000 procedures have been performed within the EPA Human Studies Facility over the past 15 years. From January 1, 1986 through December 30, 1995, 736 procedures were performed in normal nonasthmatic subjects, and these have been reported in the Journal of Bronchology 1998; 5:185-194. The overall complication rate was less than 1% (actually less than 0.1%). The most frequent complication was a low grade fever. Bronchoscopy with BAL can be associated with more significant complications, including hypoxemia, cough, wheezing, and post-bronchoscopy fever. These may be increased in asthmatic subjects, and some investigators have found hypoxemia to be more severe in asthmatics compared to normal subjects (Djukanović, R., J. W. Wilson, C. K. Lai, S. T. Holgate, and P. H. Howarth. 1991. The safety aspects of fiberoptic bronchoscopy, bronchoalveolar lavage, and endobronchial biopsy in asthma. *Am. Rev. Respir. Dis.* 143: 772-777), while others have not (Van Vyve, T., P. Chanez, J. Bousquet, J. Y. Lacoste, F. B. Michel, and P. Godard. 1992. Safety of bronchoalveolar lavage and bronchial biopsies in patients with asthma of variable severity. *Am. Rev. Respir. Dis.* 146: 116-121); Anonymous. Workshop summary and guidelines: investigative use of bronchoscopy, lavage, and bronchial biopsies in asthma and other airway disease. *J Allergy Clin Immunol* 88:808-14, 1991.;
Jarjour NN, Peters SP, Djukanovic R, Calhoun WJ. Investigative use of bronchoscopy in asthma. Am J Respir Crit Care Med 157:692-97, 1998.) Furthermore, hypoxemia is rarely severe enough to require premature termination of the procedure and can usually be treated or prevented by supplemental oxygen (Van Wyre).

Bronchoscopies are performed by experienced physicians who are either Board-certified or Board-eligible in pulmonary medicine. The physician performing the bronchoscopy is always assisted by at least one nurse familiar with the procedure. The subject's vital signs and ECG are continuously monitored during the bronchoscopic procedures and at regular intervals during the recovery phase. The procedure is immediately terminated if the subject experiences any alterations such as tachypnea, depressed respiration, tachycardia, bradycardia, abnormal rhythms or significant changes in blood pressure. As indicated earlier, a fully equipped emergency cart including a defibrillator, endotracheal intubation equipment and emergency medications (atropine, epinephrine) is available at all times. An on-site physician is always available to respond and the UNC Memorial Hospital Emergency Room is within 1/4 mile of Human Studies Facility. Before the subject is sent home, he/she receives a brief physical examination by a physician and is given phone numbers and a pager number with which to contact a physician should he/she experience any problems or have any questions.

The complication rate during medical bronchoscopy is approximately 0.08 to 0.12% for all events, including low oxygen saturation, respiratory embarrassment, myocardial infarction, or death. The medical screening of the subjects is specifically designed to exclude subjects with a history of medical problems which might put them at risk from the particle exposure and bronchoscopic procedures. Medical problems which could arise as a result of bronchoalveolar lavage and brush biopsy include the following:

Discomfort of the nose and throat is a primary risk of bronchoscopy. As outlined, the lidocaine gargle, spray and liquid is used to anesthetize these areas prior to passing the bronchoscope to reach the lower airways. The procedure will be discontinued if adequate anesthesia cannot be achieved by the means described.

Low-grade fever (38-38.5 ºC) can occur in approximately 25 % of hospitalized subjects undergoing bronchoscopy. The fever invariably resolves within 18 hours without treatment or with acetaminophen. Our experience at HSD, EPA has shown that this fever and the associated malaise can almost always be prevented by administering ibuprofen following the procedure. The subject will be asked to contact the physician who performed the procedure or one of the nurses at the Medical Station if fever persists or is higher than 38.5 ºC. As part of the standard protocol, a nurse or physician will contact the subject by telephone between 24 and 48 hours after the procedure to inquire on the general health of the subject and specifically about the presence of fever.

Cough is a common, albeit minor, problem encountered during bronchoscopy. Cough results from mechanical stimulation of cough receptors in the airway by the bronchoscope or the cytologic brush used for the brush biopsies. In addition to the discomfort, prolonged coughing with the bronchoscope in place can result in mild mechanical trauma to the vocal cords. Lidocaine solution is passed through the bronchoscope channel at the main carina and at the level of the right mainstream bronchus to suppress the cough reflex during the procedure, more can be used as needed. The procedure will be terminated if adequate cough suppression cannot be attained.

Epistaxis is caused by trauma to the nose by the bronchoscope. This condition is expected to be minor and resolve on its own. Small streaks of blood in nasal secretions may be present for up to 12 hours following the procedure. If the bleeding becomes moderate or severe during bronchoscopy, the procedure will be terminated and the bronchoscope removed. The subject's anterior nasal passage will be packed with sterile gauze to stop the bleeding. If the bleeding does not resolve with packing, the subject will be transferred to the UNC Hospital Emergency Room.

Lidocaine use presents a small risk to the subjects, as a variable fraction of the medication is absorbed through the airway mucosa. If a significant amount of lidocaine is absorbed, adverse reactions such as bradycardia, hypotension, urticarial reactions, confusion, lightheadedness, euphoria, tremors and seizures can result. We take particular precautions to avoid the possibility of overdose of topical lidocaine and limit the total amount of lidocaine instilled into the airways to a maximum of 360 mg. Also, subjects will be screened by history prior to the procedure for known allergies to lidocaine or other similar topical anesthetics.

Bronchospasm, manifested as wheezing, chest tightness or dyspnea, is a risk of bronchoscopy that is caused by stimulation of irritant receptors in the airways by the bronchoscope. This risk is minimized by 1) only allowing persons with stable mild to moderate stable asthma to participate in the study, 2) pretreatment with inhaled bronchodilator before the procedure per request of the bronchoscopy physician and 3) provisions for terminating if bronchospasm becomes evident during the procedure. Treatment for airway constriction occurring during the procedure will be given by the physicians responsible for the procedure. Administration of inhaled bronchodilator (albuterol) is all that should be required to control the symptoms. In the unlikely event that the subject requires additional treatment because the asthma attack continues, they will be transported by ambulance to the emergency room of the UNC Hospital Emergency Room.

Pneumothorax is a risk of bronchoscopic procedures such as transbronchial biopsy, peripheral lung protected brushings or peripheral lung cytology brushings. Because these procedures will not be performed, the risk of pneumothorax in this study is extremely small. There is a very small risk of a pneumothorax resulting from a endobronchial brush biopsy. The symptoms include dyspnea and chest pain. The subjects will be interviewed and examined by the bronchoscopy physician 2 hours after the procedure with these symptoms in mind. If a pneumothorax is suspected, the subject will be transferred to the UNC Hospital Emergency Room by ambulance for further evaluation and treatment.

Bleeding in the lower airway may occur from trauma caused by the bronchoscope or by brush biopsy. Bleeding is typically very minor, in the order of 1/10 cc, is not clinically significant in an otherwise healthy subject and should spontaneously resolve in a matter of minutes. Epinephrine, diluted 1/20,000 fold will be administered through the bronchoscope to the affected site if bleeding is minor to moderate. The maximum dose of epinephrine used will be 2-3 cc to prevent local tissue necrosis. The site will be monitored through the bronchoscope until the bleeding stops. If bleeding fails to resolve with epinephrine or if it is sufficiently severe to cause hemoptysis or hemoglobin desaturation, oxygen supplementation will be provided to keep arterial oxygen saturation above 90% and the subject will be transported to the NC Memorial Hospital Emergency Room by ambulance. If it becomes necessary, the subject can be intubated before transport.

The use of epinephrine to control bleeding can produce systemic effects resulting from mucosal absorption of the drug. These symptoms are transient and include headache, palpitations and tachycardia when the dose is greater than 1 mg and is given intravenously. Two cc of a 1/20, 000 dilution of epinephrine contains 0.10 mg of the drug, a dose that is very unlikely to induce any untoward effects. This is especially true when the drug is applied to the mucosa, since only a fraction is expected to be absorbed.

Bradycardia and/or hypotension may result from increased vagal nerve output provoked by coughing or Valsalva during the passage of the bronchoscope through the vocal cords and trachea. Atropine (0.6 mg iv) is prophylactically administered to the subjects before the start of the procedure to minimize this risk. The primary side effects associated with atropine administration is mild sinus tachycardia which typically resolves within 30 minutes as the drug is cleared from the subject's system. In rare cases (less than 1 percent) the sinus tachycardia is accompanied by hypotension.

Hypoxemia: Arterial blood oxygen saturation is continuously monitored during the procedure with a finger pulse oximeter. Subjects are supplemented with oxygen at a flow rate of 2 l/min via nasal cannula during the procedure. The oxygen flow can be increased to a maximum of 6 l/min if the arterial oxygen saturation falls below 93 %. If oxygen supplementation is not effective, the procedure will be discontinued immediately. Removal of the bronchoscope and treatment with inhaled bronchodilator should be sufficient to allow the subject's arterial oxygen saturation to return to normal. Under normal conditions, oxygen supplementation is ceased once the procedure is completed. However, supplementation will be continued if arterial oxygen saturation is below 93 % on room air. In

the event that low oxygen saturation persists, if the subject cannot be weaned from the oxygen supplementation, or if it falls precipitously at any time, an emergency situation will be declared and the subject will be transported immediately by ambulance to the UNC Hospital Emergency Room. These risks are minimized during the research procedure at the Human Studies Facility, in part because premedication with sedatives (midazolam) or with opiates (demerol) (as is used in medical bronchosopy) is not required for healthy research subjects. Avoiding these drugs reduces procedure risk and allows the participants to enjoy a normal day after leaving the EPA Human Studies Facility.

The risk of pneumonia as a result of bronchoalveolar lavage in the lobe involved in the procedure is less than 1%. Symptoms of pneumonia could include fever, dyspnea, persistent cough, productive cough and chest pain. The subject will be instructed to call the physician who performed the procedure or the Medical Station if these symptoms occur. The medical staff will evaluate the subject over the phone between 24 and 48 hours after the procedure for signs of pneumonia.

The subject will be urged to contact the Medical Station or the bronchoscopy physician should he/she experience any of the following symptoms: 1) Epistaxis, 2) Persistent cough, 3) Hemoptysis, 4) Chest pain, 5) Dyspnea, 6) Wheezing, 7) Sputum production, 8) Hoarseness or sore throat.

Confidentiality Risk

Risk of breach of confidentiality is minimal. For the mild asthmatic subject, it is conceivable that an employer or insurance company could learn of the diagnosis if the subject requires medical intervention as a result of participating in the study. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files which may contain the subject's name or screening number are secure in the EPA building which has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, spirometry) will be discussed with the subject and the subject will be counseled to seek treatment from his/her personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects cannot be directly identified from the samples alone.

A.4.8. **Data monitoring and analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies). Describe the provisions for monitoring the data to ensure the safety of participants. These plans could range from the investigator monitoring subject data for any safety concerns to a sponsor-based DSMB, depending on the study.

The research objective of this study is to determine if changes in epigenetic mechanisms in bronchial epithelial cells can be linked to physiological changes in healthy and mild/moderate asthmatic volunteers safely exposed to low levels of O3 and diesel exhaust. The study will follow a randomized, repeated measures design with each subject exposed to clean air, diesel exhaust and ozone. The data analysis will be focused on changes in lung function measurements, primarily FVC and FEV_1, and secondarily, HRV measurements between pre and post 2hr exposure for each exposure and lung cell composition changes. Also blood endpoints include inflammatory factors (eg IL-6), blood clotting factors (eg fibrinogen), and susceptibility factors (eg specific genotypes associated with susceptibility to air pollution).

In evaluating exposure effects of O3 and DE upon lung function, the difference between the pre-exposure and post-exposure FEV1 will be calculated and the pre-post differences for the air and the exposures will be compared utilizing repeated measures ANOVA parametric test. Clinically significant change is determined to be a 10% decrease in FEV1 measured prior to and immediately after the exposure compared to the pre exposure value. For a conservative sample size calculation, we used one sided test for a single sample under the assumption of normal distribution with Type I error of 5% and Type II error of 20%. Therefore, a ρ value of 0.05 or less will be considered significant and the proposed sample size will provide adequate (80%) power for detecting a 10% decrease in ΔFEV1.

Sample size calculation was based on the Forced Expiratory Volume in 1 Second (FEV1) in response to the O3 exposure. Data for the power calculation were obtained from results of FEV_1 (Folinsbee, Bedi et al. 1985) for a constant acute exposure to 0.3 ppm O_3 versus filtered air. In 22 subjects, the pre exposure FEV_1 was 4.608 ± 0.514 (mean $\pm$ SEM) in FA exposure and 4.656 ± 0.592 in O_3. The post exposure FEV_1 was 4.64 ± 0.476 in FA exposure and 3.856 ± 0.687 in O_3. We used 0.687 as an estimate of standard deviation after the ozone exposure and estimated 10% decrease in pre-exposure FEV1 levels to be 0.46 ml. Based on these data the required sample size for each

group is 30 subjects. We will initially recruit 30 subjects for each group (healthy and mild/moderate asthmatics) for this study to ensure the statistical power that can analyze small differences expected from low concentration exposures.

Other Exploratory Observations:
We hypothesize that subjects harboring polymorphisms in antioxidant genes will have increased responses to diesel exhaust and ozone compared to subjects who do not. In order to address this we will use collected cell samples for in vitro exposure experiments

Exploratory endpoints will include the effect of diesel exhaust and ozone on changes in: blood CBC and differential, fibrinogen and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No **_X_** Yes *If yes, check all that apply*:

a. **_X_** Names
b. **_X_** Telephone numbers
c. **_X_** Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older
d. **_X_** Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. __ Fax numbers
f. **_X_** Electronic mail addresses
g. __ Social security numbers
h. **_X_** Medical record numbers
i. __ Health plan beneficiary numbers
j. __ Account numbers
k. __ Certificate/license numbers
l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. __ Device identifiers and serial numbers (e.g., implanted medical device)
n. __ Web universal resource locators (URLs)
o. __ Internet protocol (IP) address numbers
p. __ Biometric identifiers, including finger and voice prints
q. __ Full face photographic images and any comparable images
r. __ Any other unique identifying number, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data**. Are the identifiers in A.4.9 above linked or maintained with the research data?

_X_ yes __ no

A.4.11. **Confidentiality of the data**. Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in a locked file cabinet in the Medical Station at the U.S. EPA Human Studies Facility. The Medical Station is locked when not attended by study

staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours a day for seven days a week

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

_X_ No one
__ Coordinating Center**:**
__ Statisticians**:**
__ Consultants**:**
__ Other researchers**:**
__ Registries**:**
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission**. Please check all that apply.

For electronic data stored on a desk top computer:
_X_ Secure network _X_ Password access __ Data encryption__ Password protected file(s)
__ Other comparable safeguard (describe):

For portable computing devices/external storage devices (e.g. laptop computer, PDA, CDs, memory sticks):
__ Power-on password __ Automatic log-off __ Data encryption __ Password protected file(s)
__ Other comparable safeguard (describe):

For hardcopy data (including human biological specimens, CDs, tapes, etc.):
__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office _X_ Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials**. Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples. Subjects at any time may request that their samples be no longer stored in the repository. Any analysis in progress at the time of the request or already performed prior to the request being received by the researcher will continue to be used as part of the research study. Once the researcher is notified, the subject's specimens will be destroyed.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.
- If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. This is addressed in section B.2.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects**.
Describe who will be obtaining consent (or permission) and from whom. Include discussion, as relevant, any waiting period between the initial consent discussion and obtaining consent, and steps that will be taken to minimize coercion or undue influence. If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. It is expected that the information in the consent document(s) will be communicated to participants or their LAR. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent form. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *Choose only one:*

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study topic is sensitive so that public knowledge of participation could be damaging). *Participants should be asked whether they want documentation linking them with the research and the participants' wishes will govern whether they sign the form.* Note: This justification cannot be used in FDA-regulated research. __ yes __ no __ yes __ no
Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey).
Explain.

If you checked "yes" to either (and you are not requesting a waiver in section A.5.3) consent must be obtained orally, by delivering a fact sheet, through an online consent form, or be incorporated into the survey itself. Include a copy of the consent script, fact sheet, online consent form, or incorporated document.

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**
If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected __ yes __ no

Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).*
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Subjects for U.S. EPA Studies (95-EPA-66). Typical methods for recruiting may include mass mailings, fliers and print ads. Every effort will be made to recruit women and members of racial minority groups into this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Westat recruitment office in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. If this applies to your study, please provide the following information and complete Section C.

a. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of this study will be approximately eighteen months. Subject recruitment and screening is expected to be continuous throughout the study until the intended number of subjects is reached. Scheduling constraints imposed by concurrent studies in the U.S. EPA Human Studies Division are expected to limit the rate at which subjects can be exposed to 1-2 per week.

The physical examination evaluation may take up to one hour. If the subject is eligible for the study, the individual may have up to nine more visits to the research facility over approximately 6 months. A physical examination for bronchoscopy will be performed. The training day visit will require approximately 2 hours. Exposure day (Day 1) will last approximately 6 hours. Subjects will report to the facility at no sooner than 8 AM. The morning following the exposure, the subject will return for Day 2 which is a follow-up visit and may last approximately 4 hours. Subjects will report to the facility at no sooner than 8 AM. Subjects will then return and repeat the Day 1-2 visits with the second and third exposure session. Exposure sessions will be separated by a minimum of two weeks.

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $200.00 per year, collection of subjects' Social Security Number is required (University policy—see SSN Guidance) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point. Subjects who are dismissed by the investigators for involuntary reasons after enrollment in the study but prior to completion will be paid for their participation up to that point
.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and for any procedures cancelled up to a maximum of $100. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study:
Subjects will be paid approximately $12 per hour for participation in this study. If the subjects complete all three exposures with bronchoscopy, the total compensation including prestudy qualifications will be approximately $2516.

Pre-study qualifications

recruitment screening	$15	Previously paid
physical exam	$15	Previously paid
bronchoscopy physical exam	$20	

Pre-study qualification total = $50

Training Day (approximately 2 hours)

Spirometry	$20
Time (2 h @$12/h)	$24

Training Day Total = $ 44

Subjects will be exposed to ozone, diesel exhaust and clean air in separate sessions.

Exposure Day 1 (6 hours)

venipuncture (~80ml, pre)	$ 30 per exposure
Holter monitor	$100 per exposure
chamber exposure (2 hours)	$ 72 per exposure
venipuncture (~80 ml, post)	$ 30 per exposure
spirometry (before and after exposure)	$ 40 per exposure
time (4 h @$12/h)	$ 48 per exposure
Lunch:	$ 5 per exposure

Day 1 total for completion of ONE exposure = $ 325
Day 1 total for completion of TWO exposures = $ 650
Day 1 total for completion of THREE exposures=$975

Exposure Day 2 (4 hours)

Spirometry before bronchoscopy	$ 20 per exposure
venipuncture (~80ml, 24h post)	$ 30 per exposure
time (2h @$12/h)	$ 24 per exposure
Bronchoscopy	$350 per exposure
Endobronchial Brush biopsies	$ 25 per exposure

Day 2 total for completion of ONE exposure = $449
Day 2 total for completion of TWO exposures = $ 898
Day 2 total for completion of THREE exposures=$1347

Protocol Completion Bonus $100

TOTAL for completion of ONE exposure (excluding Prestudy qualifications and training day) **$774**
TOTAL for completion of TWO exposures $1548

TOTAL for completion of THREE exposures (including completion bonus) **$2422**

If a subject begins but cannot complete the bronchoscopy procedure for voluntary or involuntary reasons, they will receive compensation for participation up to that point.

Subjects will be asked to bring a lunch for the exposure day and a refrigerator and microwave will be available. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Existing Data, Records or Human Biological Specimens

→ ***This section applies even if records are only used to identify potential subjects.***

→ ***If your study does not use existing data, records or specimens for any purpose, do not submit this section.***

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
If applicant was involved in the original collection, please explain role:

__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C.4. For each of the boxes checked in 1, do you have permission from the custodians of the data, records or human biological specimens (e.g., pathology dept, tissue bank, original researcher)? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do *all* of these data, records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 24 hrs post end Exposure (Circle one)

INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE (not present)
1 = TRACE/NOTICED (barely detectable)
2 = MILD/LIGHT (present, but not annoying)
3 = MODERATE (present, but somewhat annoying)
4 = SEVERE/HEAVY (present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4